

This listing of claims will replace all prior versions, and listings, of claims in the application:

**LISTING OF CLAIMS:**

Claims 1-32 (canceled)

Claim 33 (currently amended) An *in vitro* assay for a biological effect of presence of a protein or polypeptide or other product of cDNA expression in a mouse embryonic stem (ES) cell, a mouse embryonal carcinoma (EC) cell or a mouse embryonic gonadal (EG) cell, or a differentiated derivative thereof, comprising the steps:

- (a) transfecting the mouse cell with a first episomal vector that expresses a viral replication factor;
- (b) transfecting the mouse cell of step (a) with a second vector, wherein
  - (I) the second vector contains a cDNA coding for the protein or polypeptide or other product of cDNA expression in operative combination with a promoter for expression of the cDNA;
  - (II) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and
  - (III) the viral replication factor of step (a) replicates the second vector episomally;
- (c) isolating mouse cells of step (b); and
- (d) maintaining the isolated mouse cells over a plurality of generations, ~~so as to assay and~~
- (e) assaying the biological effect of expression of the protein or polypeptide or other product of cDNA expression,

wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate assays in which steps (b)-(d) are carried out a plurality of times with second vectors containing different cDNA sequences.

Claim 34. (canceled)

Claim 35. (currently amended) The ~~assay~~ according to Claim 33, for assay of a biological effect of simultaneous presence of a first protein, polypeptide or other

product of

cDNA expression and a second protein, polypeptide or other product of cDNA expression in a mouse ES cell, a mouse EC cell or a mouse EG cell, or a differentiated derivative thereof, further comprising the step of transfecting the mouse cell of step (b) (c) with a third vector, wherein the third vector contains

- (1) a cDNA coding for the second protein, polypeptide or other product of cDNA expression in operative combination with a promoter for expression of the cDNA, and
- (2) a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker, wherein the viral replication factor of step (a) replicates the third vector episomally.

Claim 36. (original) A method of screening a library of cDNAs comprising assaying the effect of expression of each of the cDNAs according to the assay of Claim 33.

Claim 37. (currently amended) An *in vitro* method of assaying whether a DNA under investigation codes for a polypeptide that directs transport of a cell active protein to a cell surface comprising the steps of:

- (a) expressing in a mouse cell, selected from the group consisting of an ES cell, an EC cell, an EG cell and differentiated progeny thereof, a composite DNA including (a) a DNA sequence under investigation, operatively linked to (b) a DNA coding for the cell active protein in a mouse cell, selected from the group consisting of an ES cell, an EC cell, an EG cell and differentiated progeny thereof, wherein
  - (i) activity of the cell active protein is dependent upon transport of the cell active protein to the cell surface,
  - (ii) the DNA of (b) does not code for a polypeptide that directs transport of the cell active protein to the cell surface, and
  - (iii) the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate; and
- (b) (i) if the cell is selected from the group consisting of an ES cell, an EC

cell, and an EG cell, determining whether the cell differentiates, and  
(ii) if the cell is the differentiated progeny of a cell selected from the group  
consisting of an ES cell, an EC cell, and an EG cell, determining  
whether said differentiated progeny differentiates further.

Claim 38. (currently amended) The method according to Claim 37 wherein the DNA under investigation is a library of cDNAs, which is screened to identify DNA sequence coding for signal polypeptide sequences that transport proteins to the cell surface, and wherein the method comprises the additional step of determining whether the cell active protein is transported to the cell surface and remains there or is secreted by the cell.

Claim 39. (original) The method according to Claim 37 wherein the DNA of (b) is obtained by deleting or disabling, from a DNA encoding a cell surface or secreted protein, that portion of the DNA that codes for the polypeptide sequence responsible for transportation of the protein to the cell surface.

Claim 40. (original) The method according to Claim 37 wherein the cell active protein induces a morphological or proliferative change in the cell.

Claim 41 (canceled)

Claim 42. (original) The method according to Claim 37 wherein the cell active protein is a cell surface receptor.

Claim 43. (original) The method according to Claim 42 wherein the cell active protein is an IL-6 receptor and the DNA of (b) encodes a modified form of the receptor preprotein lacking a functional signal sequence.

Claims 44-46 (canceled)

Claim 47. (currently amended) The method according to Claim 37, wherein the composite

DNA is expressed by:

- (a) (i) transfecting the mouse cell with a first vector that expresses a viral replication factor; or
  - (ii) ~~otherwise obtaining a transgenic mouse cell, selected from the group consisting of an ES cell, an EC cell, and an EG cell that expresses the replication factor;~~
- (b) transfecting the mouse cell of step (a) with a second vector, wherein:
  - (i) the second vector contains the composite DNA in operative combination with a promoter for expression of the composite DNA;
  - (ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and
  - (iii) the viral replication factor of step (a) replicates the second vector episomally;
- (c) isolating mouse cells of step (b); and
- (d) maintaining the isolated mouse cells over a plurality of generations; and
- (e) assaying so as to assay the effect of expression of the composite DNA,  
wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate methods in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.

Claim 48. (canceled)

Claim 49. (original) The method according to Claim 37 for identification of a DNA coding for a cell surface or secreted protein comprising isolating the DNA under investigation.

Claim 50. (original) The method according to Claim 37 for identification of a cell surface or secreted protein comprising isolating a protein or polypeptide encoded by the DNA under investigation.

Claims 51-54 (canceled)

Claim 55. (currently amended) The assay according to Claim 33, wherein the viral replication factor is a viral replication factor selected from polyoma large T antigen, ~~EBNA-1 antigen~~, papilloma virus replication factors, and SV40 large T antigen.

Claim 56. (original) The assay according to Claim 33, wherein said second vector contains a DNA that codes for an anti-sense RNA.

Claim 57. (currently amended) The assay according to Claim 33, further comprising the step: (e) isolating said cDNA coding for the protein or polypeptide or other product of cDNA expression.

Claim 58. (currently amended) An *in vitro* assay for a biological effect of presence of first and second products of cDNA expression in mouse embryonic stem (ES) cells, mouse embryonal carcinoma (EC) cells or mouse embryonic gonadal (EG) cells, comprising the steps:

- (a) transfecting the mouse cells with a first vector that expresses a viral replication factor;
- (b) isolating cells of step (a) and dividing the cells into at least a first sub-population of transfected cells and a second sub-population of transfected cells;
- (c) transfecting the first sub-population of transfected cells with a second vector, wherein the second vector contains a DNA coding for a first product of cDNA expression in operative combination with a promoter for expression of the cDNA and also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker, and wherein the viral replication factor of step (a) replicates the second vector episomally;
- (d) isolating cells of step (c);
- (e) maintaining the isolated cells of step (d) over a plurality of generations so as to assay the biological effect of expression of the first product of

cDNA expression:

- (f) transfected the second sub-population of transfected cells with a third vector, wherein the third vector contains a DNA coding for a second product of cDNA expression in operative combination with a promoter for expression of the cDNA and also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker, and wherein the viral replication factor of step (a) replicates the third vector episomally;
- (g) isolating the cell of step (f); and
- (h) maintaining the isolated cells of step (g) over a plurality of generations so as to assay the biological effect of expression of the second product of cDNA expression.